Original Article

Mesenchymal Stem Cell Conditioned Medium as Good as Methyl Prednisolone in Decreasing Levels of Interleukin 10 and The Degree of Pulmonary Vasculitis in Lupus Mice

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Abstract

***Background*:** Systemic lupus erythematosus is a chronic autoimmune disease that affects target organs. mesenchymal stem cell conditioned medium has immunosuppressive, anti-inflammatory, and immunoregulatory properties in lupus. Methyl prednisolone is a standard drug for lupus with immunosuppressive and anti-inflammatory properties. This study aims to compare the therapeutic effect of mesenchymal stem cell conditioned medium administration compared to methyl prednisolone on interleukin 10 levels and the degree of pulmonary vasculitis of lupus mice. ***Methods*:** The subjects were 24 female mice of Mus musculus Balb/C strain, which were categorized into 4 groups of 8 mice, i.e. the control group receiving 0.5 cc of 0.9% NaCl injection and placebo, the lupus group receiving 0.5 cc of pristane injection and placebo, and the treatment mesenchymal stem cell conditioned medium group receiving 0.5 cc pristane injection and mesenchymal stem cell conditioned medium 0,5 cc, and methylprednisolone group receiving 0,5 cc pristiane injection and methylprednisolone p.o 1,5 mg/kgbodyweight. After 24 days the mice were terminated and kidney and blood samples were taken. Statistical analysis was performed using ANOVA test followed by independent T-test. The p value was considered significant when the $p < 0.05$. ***Results*:** The study showed that there was no difference on the levels of interleukin level10 among mesenchymal stem cell conditioned medium goup and methyl prednisolone group (CM = 5,94 ± 2,49 pg/mL, mp = 5,86+1,73 pg/mL; p = 1) and the degree of pulmonary vasculitis (CM= 1,94 ± 0,25, MP=1,89+ 0,11 pg/ml; p = 0.667). Mesenchymal stem cell conditioned medium as good as methyl prednisolone in decreasing levels of interleukin 10 and the degree of pulmonary vasculitis in lupus mice. ***Conclusion*:** Mesenchymal stem cell conditioned medium as good as methyl prednisolone in decreasing levels of interleukin 10 and the degree of pulmonary vasculitis in lupus mice

Keywords: Mesenchymal stem cell conditioned medium, methyl prednisolone, interleukin 10, pulmonary vasculitis, lupus mice

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Introduction

Systemic Erimatous Lupus (SLE) is a multisystem disease caused by antibody production and deposition of complementary immune complexes which results in tissue damage.[1] The clinical manifestations of SLE are very broad, including the involvement of the skin and mucosa, joints, blood, heart, lungs, kidneys, central nervous system and immune system. Although rarely recognized, lung disease occurs in half of patients who suffer from LES. The manifestations obtained include pleurisy, vasculitis, pulmonary hypertension, and interstitial lung disease.[2] IL-10 has

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been considered as an important modulator of SLE disease activity. Patients with SLE produce large amounts of IL-10[3], where the levels in serum have a correlation with the severity of the degree of disease[4].

Pristan or Tetramethylpentadecane (TMPD) is a substance that acts to induce LES in animals. Mice with TMPD injection show a clinical picture of lupus such as arthritis, and pulmonary capillaritis. Administration of pristan in BALB / c mice triggers autoantibodies as a characteristic of lupus. Mice exposed to pristan also have immune complex deposits in the kidneys which cause severe proteiuria and nephritis. Mice by TMPD injection met 4 1997 ACR criteria for LES enforcement, namely dsDNA antibodies, arthritis, lupus nephritis, and vasculitis[5].

The pathogenesis of the disease that is still unclear and the therapy that is given less than optimal can result in high SLE mortality. The current SLE therapy is only to inhibit progression and prevent the severity of the disease. The absence of definitive cure therapy for SLE has made many research breakthroughs in the treatment of SLE. Conditioned Media mesenchymal stem cells have been shown to reduce the degree of kidney damage, the degree of lung damage, dsDNA antibodies, and C3 complement in lupus model mice[6] however, there has never been a comparison with standard SLE therapy

Mesenchymal stem cells are one of the promising new therapies for SLE. The secretion of conditioned media from mesenchymal stem cells (secretome) containing cytokines, micro RNA (miRNA), exosomes and microvesicles will have a therapeutic effect[7] and not due to cell differentiation[8]. Mechanisms of this secretome include antiapoptotic[9], anti-inflammatory[10], anti-fibrotic[11], angiogenic[12] and tissue regeneration effects.

Our study was aimed to compare the therapeutic effect of mesenchymal stem cell conditioned medium administration compared to methyl prednisolone on interleukin 10 levels and the degree of pulmonary vasculitis of lupus mice.

Methods

The present study was an experimental study using a design of post-test only control group. The objects of the study were 21 female mice of Mus musculus species Balb/C line aged 3-4 months, weighed 20-30 gram, which were categorized into three groups. The initiation phase by inducing the mice using 0.5 cc intraperitoneal injection of pristane within 3 weeks that would induce a clinical manifestation of lupus disease in the mice. After that, therapy was given for 2 weeks. The lupus group received 00.5 cc pristane intraperitoneal injection at the initation of treatment and 0.2 cc 0.9% NaCl orally on 3 weeks later; while the mice in the conditioned media mesenchymal stem cells group were injected with 0.5 cc pristane intraperitoneal injection at the initation of treatment and 0.45 cc secretome mesenchymal stem cells on 3 weeks later. Those in the standart treatment group received 0.5 cc pristane intraperitoneal injection at the beginning of treatment and methyl prednisolone dose 1,5 mg/kg orally on 3 weeks later. After 5 weeks, the mice were killed, blood samples were withdrawn, right kidney were obtained and subsequently the levels of IL 10 and lung biopsy of all mice in treatment group were evaluated.

The evaluation of IL 10 was performed using immunohistochemistry; while histological examination was carried out using hematoxylin and eosin staining on kidney and The histological features of lung in the mice were evaluated by scoring perimeter score. Perimeter scores indicate the percentage of perimeter vessels surrounded by cells. Score 1 represents 5-24% perimeter of the veins surrounded by cells, 25–49% of veins surrounded by infiltration receive a score of 2. Score 3 is given for 50-74% and 4 for 75-100% of veins surrounded by cells . The depth of the inflammatory infiltration was also measured. The score reflects the appearance of mice pulmonary vasculitis.

The obtained data were expressed in mean value + standard deviation. Normality test was performed using Shapiro-Wilk test and variant homogenicity test was performed using Levene's test, F Anova test followed by Least Significant Difference (LSD) post-hoc test for data with normal and homogenous distribution and Kruskal-Wallis followed by Mann-Whitney test for data with abnormal or non-homogenous distribution; while regression analysis was carried out to identify the variable which was most affected by secretome. The significance level was at $p< 0.05$.

Results

1. Description of the results variable

The mean results of the IL 10 variables and pulmonary vasculitis scores in the Lupus group, the conditioned media (CM) mesenchymal stem cell group, and the standart therapy group are as described below.

2. Effect of CM mesenchymal stem cells and Methyl prednisolone in Pristan-Induced Mice

Table 1. Description of the results variable

Variable (Mean±SD)	Lupus	CM stem cells	Standart Treatment
Interleukin 10	10.91 +3.17	5.94 +2.49	5.86 +1.73
Pulmonary vasculitis	2,43 ± 0,24	1,94 ± 0,25	1,89 ± 0,11

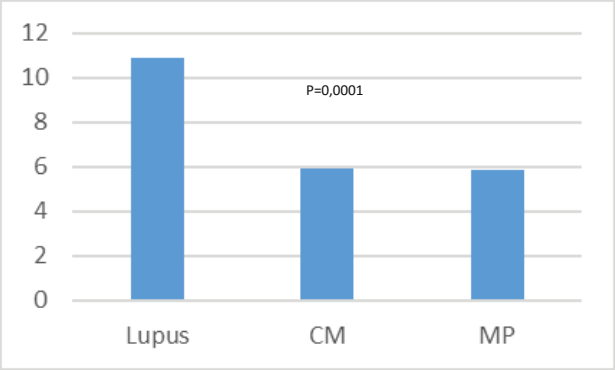

Figure 1. Effect of CM mesenchymal stem cells and Methyl prednisolone on Interleukin 10 Levels in Pristan-Induced Mice. The study showed that there was a difference on the levels of IL 10 level among the three groups (Lupus 10.91 +3.17, CM= 5.94 +2.49, standart treatment = 5.86 +1.73; p = 0.0001).

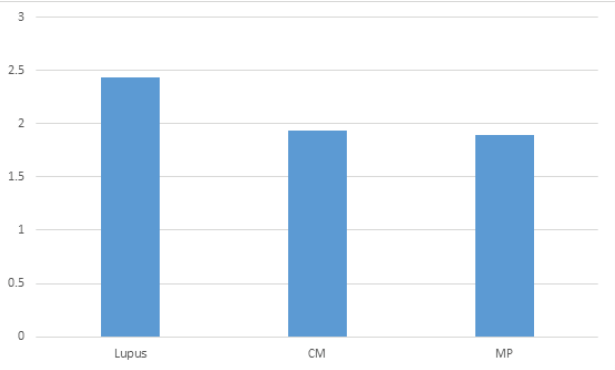

Figure 2. Effect of CM mesenchymal stem cells and Methyl prednisolone on pulmonary vasculitis in Pristan-Induced Mice. The study showed that there was a difference on the levels of Pulmonary vasculitisl among the three groups (Lupus = 2,43 ± 0,24,CM= 1,94 ± 0,25, standart treatment = 1,89 ± 1,54 pg/mL; p = 0.001).0,11

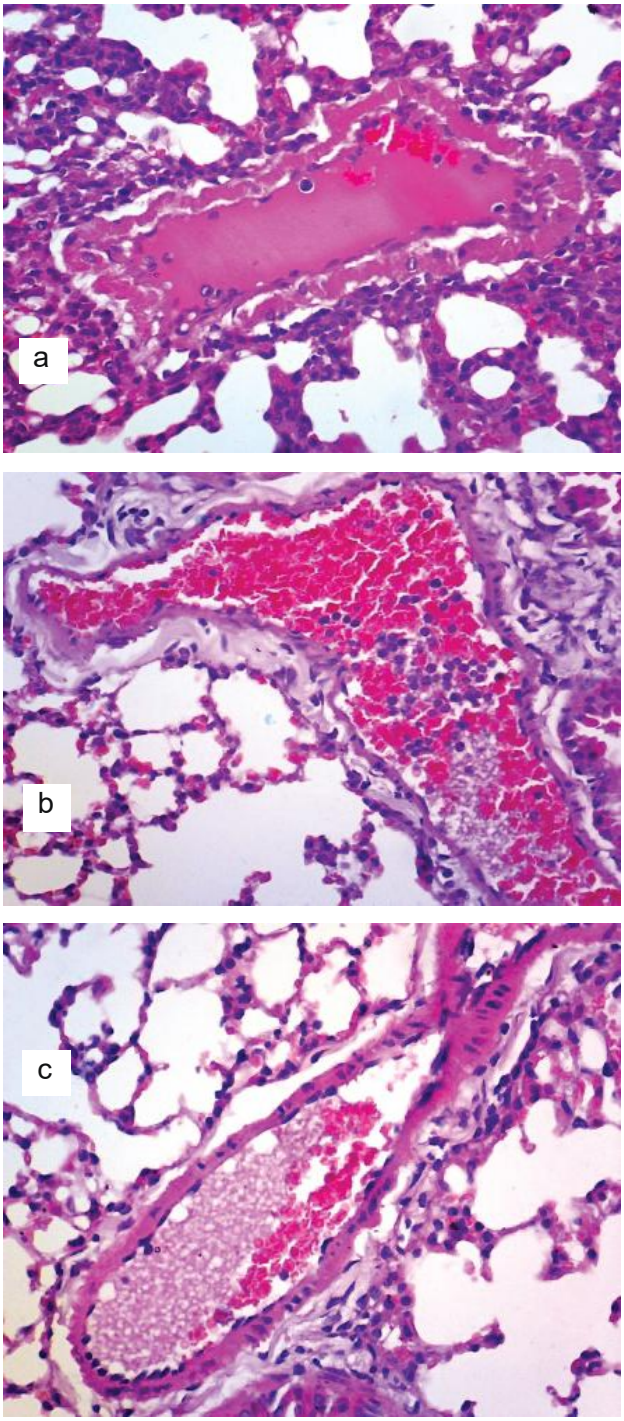

Figure 3. Overview of Histology of Lung Vasculitis

2.1. Effect of CM mesenchymal stem cells and Methyl prednisolone on Interleukin 10 Levels in Pristan-Induced Mice

The results of the analysis of the difference in 2 independent sample means using the Post Hoc Bonferroni Test showed that the test of difference in the IL-10 variable between the Pristan Treatment group with the treatment group pristan and secretome was significant with a p-value = 0.005 (p <0.05). It can be said that in mice after administration of scretome therapy had a lower average IL-10 (decreased) significantly compared to the lupus group. Then the mice after being given Methylprednisolone therapy the average IL-10 variable was also lower (decreased) compared to the lupus group with a p-value = 0.005 (p <0.05).

The results of this study note that the low (decreased) average IL-10 levels due to pristan and scretome therapy turned out to be slightly higher than the average IL-10 levels in the pristan and Methylprednisolone treatment groups with p-value = 1,000 (p> 0.05) which means that there is no significant difference. It can be interpreted that the average IL-10 level due to pristan and secretome therapy is the same as with an average level of IL-10 in the treatment groups of pristan and Methylprednisolone.

Figure 3. Overview of Histology of Lung Vasculitis

A. Pristan control group (Lupus); B. Pristan treatment group with secretome / CM messenchymal stem cells; C. Pristan and Methylprednisolone Treatment Groups (standard therapy).

Discussion

The mesenchymal stem cell secretome inhibits excessive T cell proliferation thereby suppressing IL-10 production and inhibiting autoreactive B cell hyperactivity. The formation of autoantibodies is suppressed so as to prevent the buildup of immune complexes in renal mesangial cells and prevent further kidney damage. IL-10 plays an important role in the pathogenesis of SLE because of its ability to stimulate the proliferation and differentiation of B cells to form autoantibodies. IL-10 is positively correlated with SLE disease activity, inhibits Th1 cells and cellular immunity resulting in impaired immune regulation in SLE[1,7,13]. IL-10 which is elevated in SLE is proinflammatory especially those related to Th2 responses, specifically B cells, granulocytes and natural killer cells. IL-10 in SLE patients is higher than in healthy people and is in harmony with disease activity. Excessive IL-10 production can cause immunosuppression and especially in humans will cause various complications due to uncontrolled humoral responses[14].

The incidence of vasculitis in patients with LES can be cutaneous or visceral. Lung vasculitis is one of the features of visceral vasculitis in LES. In this study the degree of vasculitis was measured histologically by assessing inflammatory cells in the pulmonary arteries. The results of this study showed a decrease in the degree of vasculitis in the lungs after mesenchymal stem cell secretome therapy. This study is similar to a study by Sun et al in 2009, in which Sun compared lupus model mice with bone marrow mesenchymal stem cell therapy with cyclophosphamide therapy. The results showed that bone marrow mesenchymal stem cell therapy showed a decrease in the degree of vasculitis in the liver blood vessels, where there was a decrease in inflammatory cells in the liver porta vein when compared with cyclophosphamide therapy[15].

In this study the administration of methylprednisolone has not been able to change the level of interleukin 10, even the administration of methylprednisolone makes IL-10 levels lower. Glucocorticoids are extensively used for inflammatory conditions. However, glucocorticoid resistance occurs in some patients. Several mechanisms have been proposed to underlie glucocorticoid resistance and more recently glucocorticoid sensitivity differs from the Th subset it has been suggested to underlie different glucocorticoid sensitivity from different subsets of patients. Th1 and Th2 cells are sensitive to glucocorticoid inhibition while Th17 cells are resistant to glucocorticoid suppression. However, Th17 cells in certain diseases such as psoriasis appear to be sensitive to glucocorticoid inhibition while Th17 cells in some other diseases such as Crohn's disease are resistant to glucocorticoids.[16]

Conclusion

The study demonstrates the effect of moringa Mesenchymal stem cell conditioned medium as good as methyl prednisolone in decreasing levels of interleukin 10 and the degree of pulmonary vasculitis in lupus mice.

Ethical clearance

Ethical clearance was sought from the Ethical Review Board from Medical Faculty of Sebelas Maret University

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Conflict of interest

The author declares that they have no conflicts of interest

Author's contribution

Data gathering and idea owner of this study, Study design, Datagathering, Writing and submitting manuscript, Editing and approval of final draft, all events done by all the authors.All authors read and approved the final manuscript.

References:

1. Tutuncu, Z., Kalunian, K. The Definition and Classification of Systemic Lupus Erythematosus. In : Wallace DJ, Bevra HH. DUBOIS Lupus Erythematosus and Related Syndromes. Eighth Edition. Philadelpia : Saunders; 2013.. Pp : 25-35
2. Mitto S, Fell CD. Pulmonary Manifestations of Systemic Lupus Erythematosus. *Semin Resp Crit Care Med*. 2014;**35**(2):249-254. https://doi.org/10.1055/s-0034-1371537
3. Llorente L., Richaud-Patin Y., Couderc J., Alarcon-Segovia D., Ruiz-Soto R., Alcocer-Castillejos N., Alcocer-Varela J., Granados J., Bahena S., Galanaud P., Emilie D. Dysregulation of interleukin-10 production in relatives of patients with systemic lupus erythematosus. *Arthritis Rheum*. 1997;**40**:1429-1435M https://doi.org/10.1002/art.1780400810
4. Moon KM, Park YH, Lee JS, Chae YB, Kim MM, Kim DS, et al. The effect of secretory factors of adipose-derived stem cells on human keratinocytes. *Int J Mol Sci*. 2012;**13**(1):1239-57. https://doi.org/10.3390/ijms13011239
5. Reeves W.H., Lee P.Y., Weinstein J.S., Satoh M., Lu L. Induction of autoimmunity by pristane and other naturally occurring hydrocarbons. *Trends Immunol*. 2009; **30**(9): 455-464. https://doi.org/10.1016/j.it.2009.06.003
6. Nurudhin Arief. Effect of messenchymal stem cell secretome on levels of anti ds DNA antibodies, C3 complement, pulmonary histology and histologic features of rats induced by pristan. 2017. Dissertation
7. Madrigal M, Rao KS, Riordan NH. 2014. A review of therapeutic effects of mesenchymalstem cell secretions and induction of secretorymodification by different culture methods. *Journal of Translational Medicine*;**12**:260 https://doi.org/10.1186/s12967-014-0260-8
8. Bi XY, Zhang HC, Liu XB, Huang S, Wang HX, Xie LX, et al. 2012 Microvesicles derived from human umbilical cord mesenchymal stem cells stimulated by hypoxia promote angiogenesis both in vitro and in vivo. *Stem Cells Dev*; **21**(18):3289-3297. https://doi.org/10.1089/scd.2012.0095
9. Shabbir A, Zisa D, Suzuki G, Lee T. 2009. Heart failure therapy mediated by the trophic activities of bone marrow mesenchymalstemcells:anoninvasivetherapeuticregimen. Am J Physiol Heart Circ Physiol ; 296(6):H1888-H1897. https://doi.org/10.1152/ajpheart.00186.2009
10. Bartosh TJ, Ylöstalo JH, Mohammadipoor A, Bazhanov N, Coble K, Claypool K. 2010. Aggregation of human mesenchymal stromal cells (MSCs) into 3D spheroids enhances their antiinflammatory properties. Proc NatlAcad Sci USA;107(31):13724-13729. https://doi.org/10.1073/pnas.1008117107
11. Mirotsou M, Jayawardena TM, Schmeckpeper J, Gnecchi M, Dzau VJ. Paracrine mechanisms of stem cell reparative and regenerative actions in the heart. *Journal of Molecular and Cellular Cardiology.* 2011 Feb;**50**(2):280–9.
12. Kinnaird T, Stabile E, Burnett MS, Epstein SE. 2004. Bone-marrowderived cells for enhancing collateral development: mechanisms, animal data, and initial clinical experiences. Circ Res 20;95(4):354-363. https://doi.org/10.1161/01.RES.0000137878.26174.66
13. Meirelles L, Fontes AM, Covas DT, Caplan A. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. *Cytokine Growth Factor Rev*. 2009 Oct-Dec;**20**(5-6):419-27. https://doi.org/10.1016/j.cytogfr.2009.10.002
14. Bijjiga E, Martino AT. Interleukin 10 (IL-10) Regulatory Cytokine and its Clinical Consequences. *J Clin Cell Immunol*. 2013. **S1**:007. doi:10.4172/2155-9899.S1-007 https://doi.org/10.4172/2155-9899.S1-007
15. Sun L, Akiyama K, Zhang H, Yamaza T, Hou Y, Zhao S, et al. Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic
16. lupus erythematosus mice and humans. Stem Cells 2009. 27:1421-1432. https://doi.org/10.1002/stem.68
17. Banuelos J, Lu N. A gradient of glucocorticoid sensitivity among helper T cell cytokines. *Cytokine Growth Factor Rev*. 2016 Oct; **31**: 27-35. https://doi.org/10.1016/j.cytogfr.2016.05.002